Successful pregnancy after etanercept immunotherapy in women with a history of recurrent miscarriage

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Introduction. The aim of the study was to determine pregnancy outcome after etanercept immunotherapy in women with a history of at least three recurrent miscarriage (RM) or failed IVF. Etanercept is a tumor necrosis factor antagonist with anti-inflammatory effects. Its major mode of action is to suppress TNF-alpha, a Th-1 embryotoxic cytokine produced by activated natural killer (NK) cells.
Materials and Methods. We studied pregnancy outcome in 25 women with increased NK cell number and/or activity before conception. Women received 4 doses (25 mg) of etanercept twice weekly before conception. The consent for the study from the Bioethics Committee of the Military Institute of Health Sciences and from the patients was obtained. Natural killer cell activity was measured using flow cytometry. In addition, the following peripheral blood NK cells' surface antigens: CD16, CD56 were studied using flow cytometry, before treatment and 2 weeks after the last etanercept dose.
Results. We determined that there is a positive correlation between decreased natural killer cell activity after etanercept therapy and successful pregnancy in the study women ($r>0.5$, $P<0.05$).
Conclusions. Etanercept therapy might be effective treatment for women with increased NK cell activity. Regulation of immune system activity may underlie possible effect of such therapy.

Human chorionic gonadotropin beta gene variants and expressional profile are associated to recurrent miscarriages.

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The recurrent miscarriage (RM, ≥3 consecutive pregnancy losses) affects 1-2% of fertile couples. In >50% cases the cause of RM remains unknown. The genetic factors may contribute in the pathogenesis of pregnancy loss. One of the first proteins synthesized by the conceptus is human chorionic gonadotropin (hCG). Besides its luteotropic function hCG regulates implantation and immunomodulation at maternal-fetal interface. Low level of hCG is related to miscarriage. Critical for hCG function is the beta-subunit of the hormone coded by four genes: CGB, CGB5, CGB7, CGB8, sharing the common gene cluster with highly homologous LHB and two beta-subunit non-coding CGB genes.

Objectives: The aim of the study was to (i) determine the expression profile of all CGB genes during the normal and failed pregnancy; and (ii) find variants of hCG beta genes that are related to RM.

Methods: The expression of CGB genes in trophoblastic tissue from normal and complicated pregnancy (RM, ectopic pregnancy-EP) was determined by sensitive real-time PCR; and semi-quantitative RT-PCR GeneScan analysis that allows the discrimination of each individual gene. CGB5 and CGB8, as the most hormone production contributing genes were fully resequenced in Estonian and Finnish RM patients (n=184) and fertile women (n=195).

Results: In cases of RM, the transcription of CGB genes was low indicating its possible involvement in the pathogenesis of miscarriage. In EP, CGB genes were highly expressed in contrast to the low hormone concentration suggesting the other mechanisms than the pathological variation and low transcriptional activity of hCGbeta genes dominate. The most prevalent expression pattern of hCGbeta genes was CGB8>CGB5≈CGB>>CGB. Significant protective effect was associated with two SNPs located at identical positions in intron 2 in both CGB5 ($p=0.007$, OR=0.53) and CGB8 genes ($p=0.042$, OR=0.15); and four SNPs located in promoter area of CGB5 ($p<0.03$; OR=0.54-0.58). All these SNPs occur more frequently in fertile women (MAF 12.05%-14.36%) compared to the RM patients (7.10%-8.15%).

The haplotype structure of the CGB8 promoter differed from the corresponding region in CGB5 being consistent with balancing selection untolerating de novo alterations. One of three SNPs in 5'upstream region of CGB8 located in initiator element critical for transcription; and three rare non-synonymous amino acid substitutions were identified only among the RM patients.

Conclusions: RM is associated with low transcriptonal activity of all CGB genes. The promoter and intronic variants of CGB5 carring the protective SNPs decrease the risk of RM ~1.7-fold, rare variants in CGB8 increase the risk for pregnancy loss. The data on

expression profile in trophoblastic tissue and higher conservation of CGB8 compared to other hCGbeta genes suggests its critical role in reproductive success.The diagnostic application of our findings may be useful in the management of RM.

Patients' preferences for salpingostomy relative to salpingectomy in tubal ectopic pregnancy: a discrete choice experiment

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Introduction: Whether tubal ectopic pregnancy (EP) should be treated by laparoscopic salpingostomy or by salpingectomy from the perspective of future natural conception chances, is subject of ongoing debate. It is unknown which treatment women with a desire for future pregnancy prefer in view of the possibly better fertility outcome after salpingostomy and the potential disadvantages of this treatment, i.e. persistent trophoblast (PT) needing additional methotrexate treatment and an increased risk for repeat EP. We investigated patients' preferences for laparoscopic salpingostomy relative to laparoscopic salpingectomy by means of a discrete choice experiment (DCE) and compared these to preferences in a group of non pregnant subfertile women desiring future pregnancy

Methods: The patients were women surgically treated for tubal EP as part of an ongoing randomised controlled trial (ESEP study, ISRCTN37002267). A group of subfertile women desiring pregnancy was also invited to participate in the study. This group, comprising consecutive new patients visiting our infertility clinics, was considered to be a representative of those who might be faced with an EP in the future. All women were offered a web-based DCE of 16 choice sets. Each choice set consisted of two profiles representing hypothetical scenarios of salpingostomy. An 'opting out' option, representing the values in case of salpingectomy, was the same for every choice set. A conditional logistic regression model was used to analyse relative importance of the attributes.

Results: We included a total of 94 women (51 patients and 43 subfertile women). Women surgically treated for EP were willing to accept the risk of a repeat EP only if this was compensated by a 2.1 fold increased pregnancy rate, whereas subfertile women did this at an 1.6 fold increase in pregnancy rate. Subfertile women had a baseline preference for salpingostomy if the clinical outcome was the same as for salpingectomy. For all respondents the risk of PT was acceptable if compensated by a small rise in the spontaneous pregnancy rate.

Conclusions: The risk of a repeat EP after treatment strongly influences women's preferences for laparoscopic salpingostomy relative to laparoscopic salpingectomy for tubal EP. Women who had been surgically treated for an EP weighed the risk of a repeat EP stronger than subfertile women at risk for EP.

Pre-implantation genetic screening (pgs) as a tool to increase live birth rates in couples with unexplained recurrent miscarriage

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Introduction

PGS has been advocated as a promising technique to improve live birth rates and decrease miscarriage rates in couples with unexplained recurrent miscarriage (RM).The rationale behind the use of PGS in case of unexplained RM is that aneuploidy of the embryo may be the cause of the recurrent miscarriage. However, whether PGS is really beneficial in these couples remains controversial especially since several recent trials have shown a detrimental effect of PGS on live birth rates in subfertile women with advanced maternal age. The aim of this study was to review the literature with respect to live birth rates after PGS and after natural conception in couples with unexplained RM to determine whether PGS is useful in these couples.

Material & methods

MEDLINE, EMBASE and CENTRAL databases were searched untill April 2008. Unexplained RM was defined as two or more miscarriages without an identified underlying cause. We searched for randomized control trials and/ or comparative studies comparing PGS with natural conception in couples with unexplained RM and cohort studies or randomised studies in which PGS or natural conception were compared to another intervention.As PGS cycles are usually completed within a restricted time frame, we divided the natural conception studies into two groups; one group with a time to horizon of one year –to allow for comparison with PGS cycles- and the other group with a time to horizon exceeding one year.

Primary outcome measure was live birth rate per couple. Secondary outcome measure was miscarriage rate per couple.

Results

We found no randomized controlled trials or non randomized comparative studies with a head to head comparison of PGS with natural conception. The search for PGS resulted in 159 titles. No randomized studies or non randomized comparative studies in which PGS was compared to another intervention in couples with RM were found. Four observational studies dealing with 181 couples were found (average 1,3 cycles per couple). Live birth rates varied between 19% and 46% (median 43,1%) and miscarriage rates between 0% and 10% (median 8,6%) per couple.

The search for natural conception resulted in 2056 publications of which 10 studies could be included. Both randomized studies and cohort studies in which natural conception was compared to another intervention were found. A total of 1418 couples with unexplained recurrent miscarriage who had been practicing natural conception were included.

Six studies had a time-horizon of one year and these studies described a total of 228 couples. Live birth rates ranged from 11% to 48% (median 48%), and miscarriage rates

ranged from 14% to 42% per couple (median 21,2%).
Four studies had a time-horizon exceeding one year and these studies described a total of 1190 couples. Live birth rates ranged from 40% to 55% (median 40%), and miscarriage rates ranged from 9% to 18% per couple (median 18%).

Conclusion

Current published data do not show substantial differences in live birth and miscarriage rates after PGS or natural conception in couples with unexplained RM. Thus, no conclusions can be drawn on the efficacy of PGS compared to natural conception in couples with unexplained RM. Nevertheless, in light of the established detrimental effect of PGS in subfertile couples of advanced maternal age, the costs associated with PGS, the morbidity associated with IVF-PGS, and the confounding effects of chromosomal mosaicism, it is our view that natural conception should be the “treatment” of preference for couples with unexplained RM.

Autoimmune and thrombophilic causes of implantation failure in greek couples

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Introduction: This was a retrospective study performed in order to investigate the distribution of various autoimmune and thrombophilic causes in the Greek population that may be associated with implantation failures (IF), the latter considered after at least three IVF failures with good embryos in healthy women below 35 years of age.

Materials and Methods: We included in this study the last 30 couples that entered our Recurrent Pregnancy Unit which had at least three IVF failures with good embryos with healthy women below 35 years of age. They had been checked for: phospholipid autoimmunity (anticardiolipins, β2GPI and serine antiphospsholipid antibodies (IgG and IgM)), ANA, antithyroid antibodies (AA), immunoglobulins (IgG, IgA and IgM) and for thrombophilia incuding protein C, protein S, activated protein C resistance (APC-R), antithrombin III, homocystein, lupus anticoagulant and the following gene mutations: FV Leiden , Prothrombin 20210A, MTHFR C677T (homozygous or double heterozygous for C677T and A1298C), and PAI-1 4G/5G. In all couples, cultures for mycoplasma hominis, ureaplasma urealyticum and chlamydia trachomatis were taken. 14 couples had been caryotyped and all couples had hysterosalpingography or hysteroscopy.

Results: At least one thrombophilic factor was found in 15 patients (50%), an autoimmune factor in 3 (10%), AA in 4 (14%), increased IgG in 1 (5%), vaginal infection in 1 (5%), abnormal karyotype in none (0%), abnormal anatomy in none (0%).

Conclusion: The distribution of the above factors is discussed along with their possible role in IF. The number of patients is small. This is a pilot study. Numbers will increase soon, though, and more definite conclusions can be made about the possible involvement of immunological factors in IF. The high percentage of patients with a thrombophilic factor is probably due to the small number of patients but, although the cost is high, it has to be taken into consideration in the work-up of these patients.

Determination of outcome in very early intrauterine pregnancies of uncertain viability

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Introduction

An intrauterine pregnancy of uncertain viability (IPUVI) may represent a normal early pregnancy of approximately 4-6 weeks gestation or a failed or failing pregnancy with arrested growth, which is destined to miscarry. Prediction of outcome in such cases is challenging. The aim of our study was to examine a large and unselected cohort of women with an IPUVI in order to determine whether factors could be identified in the history, demographics or ultrasound variables to predict outcome (at initial follow up and at the end of the first trimester), without recourse to further biochemical or ultrasound investigation.

Methods

This was a prospective observational cohort study in an early pregnancy unit involving 493 women with an empty gestation sac (GS) <20mm, GS <25mm containing yolk sac (YS) only or fetal pole <6mm without fetal heartbeat (FH) at transvaginal ultrasound. Univariate and multivariate logistic regression analysis were performed to predict ongoing pregnancy viability. Main outcome measures were viability at initial 7-14 day follow up and at 11-14 weeks.

Results

Outcome at 7-14 days was viable in 307/493 (62.3%) and miscarriage in 186/493 (37.7%). To predict viability, only gestational age (GA) less than 42 days (OR 6.85), vaginal bleeding (OR 0.27), presence of YS (OR 3.05), mean YS diameter (OR 0.08) and the difference between the mean GS and mean YS diameter (piecewise effect: <7 mm: OR 1.52; >= 7 mm: OR 0.83) were significant in multivariate logistic analysis. A model for prediction of viability produced an area under the curve (AUC) of 0.88 in a training set and 0.81 in a test set. A simplified model, which will be displayed, using only GA, vaginal bleeding and mean YS diameter as binary variables produced an AUC of 0.90 in a training set and 0.79 in a test set.

Conclusions

Although definitive prediction of outcome in IPUVI is unlikely to be possible, it is helpful to give an indication to women of the likely outcome in order to prepare them for a possible poor outcome at subsequent scans or to reduce anxiety if the outcome seems likely to be good. This study suggests that a mathematical model is effective for predicting outcome. In addition, a ‘simple rules’ model incorporating binary variables alone (gestational age less than 42 days, presence of bleeding and a visible yolk sac with

mean diameter less than 4.6mm) allows an accurate estimation of viability in the clinic setting from a straightforward chart of ‘yes or no’ questions. Application of this model has the potential to be useful in counselling women in whom the ongoing viability of an early intrauterine pregnancy is unclear, by enabling an individualized probability to be given of the likelihood of the pregnancy being successful both at initial follow up and to the end of the first trimester.

The addition of "consecutive" to the definition of recurrent miscarriage; is there any influence on the probability of carrier status?

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Introduction

Currently, no consensus exists among international guidelines regarding a clear definition of recurrent miscarriage (RM). Definitions very often contain the word "consecutive". This addition is based on doctor's beliefs and non evidence-based literature. It is unknown whether the sequence of preceding pregnancies is an important determinant of chance of underlying causes and prognosis. Especially the relation between the sequence of preceding pregnancies and the risk of parental structural chromosome abnormalities is unknown.

Recently, maternal age, number of miscarriages and family history of RM have been identified as independent factors influencing the probability of carrier status and a model for selective karyotyping has been developed[1]. The aim of our study was to investigate whether the probability of carrier status in couples with consecutive RM was different from the probability among couples with non-consecutive preceding miscarriages.

Material & methods

We performed a case-control study in six centres in the Netherlands. We included 705 couples with at least two preceding miscarriages; 278 carrier couples and 427 control couples. Information on obstetric history was obtained through medical records and patient questionnaires. We used logistic regression to analyse whether the presence of consecutive RM increased the risk for carrier status. Subsequently, multivariable logistic regression analysis was performed, taking into account known independent risk factors for carrier status.

Results

The majority of all couples (637/705) had experienced at least two consecutive miscarriages prior to chromosome analysis, 256 of 278 (92%) carrier couples and 381 of 427 (89%) non-carrier couples respectively.

There were 386 of 705 (55%) couples with a history of at least three miscarriages. The relative proportion of at least three consecutive miscarriages among these couples with at least three miscarriages was the same in both groups; 132 of 170 (78%) carrier couples and 168 of 216 (78%) non-carrier couples.

After multivariate logistic regression analysis, a history of at least two consecutive miscarriages was not an independent factor influencing the probability of carrier status (OR 0.81, CI 0.42-1.58). A history of three consecutive miscarriages even seemed to be a factor predicting a lower chance of being a carrier compared to a history of three non-consecutive miscarriages (OR 0,56, CI 0,28-1,1).

Conclusions

Among couples with recurrent miscarriage, presence of consecutive miscarriages does

not increase the probability of carrier status. Here we state that the addendum "consecutive" should be removed from any definition of RM. Patients with consecutive miscarriages or miscarriages interspersed with healthy child(ren) should not be treated differently with regard to offer or withhold parental chromosome analysis.

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